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Prenatal TCDD and predisposition to mammary cancer in the rat

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Prenatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was investigated for its potential to predispose to breast cancer. Analysis of mammary gland differentiation and cell proliferation were used as biomarkers. Timed pregnant Sprague–Dawley CD rats were gavaged with 1 μg TCDD/kg on day 15 post-conception. Control animals were treated with the same volume of vehicle (sesame oil) on the same schedule. Mammary gland differentiation studies revealed that prenatal TCDD treatment, as compared with sesame oil treatment, resulted in significantly more terminal end buds and fewer lobules II in 50-day-old offspring, but no significant alterations to mammary gland differentiation in 21-day-old offspring. Terminal end buds are the most susceptible terminal ductal structures and lobules the least susceptible to carcinogenesis. Prenatal TCDD treatment did not alter labeling index in the mammary terminal ductal structures of 21- and 50-day-old rats, but the total proliferative compartment in terminal end buds of 50-day-old rats was larger. Prenatal TCDD treatment resulted in an increased number of chemically induced mammary adenocarcinomas in rats. TCDD delayed time of vaginal opening and caused disruption to the estrous cycle. Alteration to mammary gland differentiation (increased number of terminal end buds) is correlated with increased susceptibility to mammary cancer from prenatal exposure to TCDD.

Introduction

Inherited genetic risk of breast cancer accounts for no more than 10–15% of all cases, leaving 85% of cases diagnosed among women who are not in this high risk subgroup of the population (1). Lifestyle and environmental factors must play important roles in predisposition for breast cancer. Environmental chemicals, including dioxin, have been implicated as reproductive toxins in humans, including potential for breast cancer (2), but the epidemiological reports are not in agreement. Analysis of chemical plant workers found a >2-fold increase in breast cancer in female workers exposed to dioxin contamination (3). In contrast, another 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) study was negative (4). Other studies with organochlorines also yield differing results (5).

Abbreviations: BrdU, bromodeoxyuridine; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DMBA, dimethylbenz[*a*]anthracene; TEBs, terminal end buds; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TD, terminal ducts

In animal models, TCDD given just 3 days prior to administration of dimethylbenz[*a*]anthracene (DMBA) lengthened the time required for development of DMBA-initiated mammary tumors (6). TCDD administered to adult rats having DMBA-induced mammary tumors has been reported to inhibit the growth of these tumors (7). Most of the studies have been carried out with adult or life-time exposures to TCDD. No investigations have been carried out on the potential of early exposure to TCDD to alter development and predisposition to mammary cancer. We propose this as an opposing mechanism to chemicals that can influence development and render the adult less susceptible to chemically induced mammary cancer (8–15). The latter parallels the influence of early pregnancy on mammary gland differentiation and reduced incidence of mammary cancer.

TCDD can bind to the aryl hydrocarbon receptor and function as a signal transducer and transcription factor (16–20). It also alters a wide range of estrogen-induced responses in cell lines and rodents, including cell proliferation (21–23). Treatment of MCF-7 cells with TCDD alters nuclear estrogen receptor levels (24,25). In mice, it has been shown to decrease hepatic and uterine estrogen receptor levels (7). TCDD has been reported to stimulate 17β-estradiol hydroxylase in MCF-7 cells (21), perhaps leading to the disappearance of estrogen. *In utero* and lactational exposure of rats to TCDD lowers estrogen levels and increases uterine, ovarian and hypothalamic estrogen receptor mRNA levels (26). Gray and Ostby (27) have reported that prenatal TCDD treatment on day 15 of gestation resulted in puberty being delayed. Estrous cycle-mediated running wheel activity and female sexual behavior at proestrous were not affected by gestational exposure to TCDD. TCDD alters medial epithelial cell differentiation during palatogenesis (28,29) and induces terminal differentiation of thymic epithelial cells, preventing thymocyte maturation (30).

We (12–15,31) and others (32) have demonstrated that hormonally active chemicals can alter mammary gland development. Diethylstilbestrol, DDT and genistein given prepubertally enhanced mammary gland development and stimulated cell proliferation, presumably acting as estrogens (31). Diethylstilbestrol exposure during gestation resulted in increased incidence and multiplicity of DMBA-induced mammary tumors (32). When diethylstilbestrol was given during the neonatal period only, it resulted in reduced incidence and multiplicity of chemically induced tumors (15). Also, neonatal and prepubertal exposure to the phytoestrogen genistein protected against DMBA-induced mammary cancer in rats (13,14). Neonatal and prepubertal genistein treatments caused early gland differentiation [fewer terminal end buds (TEBs) and more lobules]. On the other hand, TCDD given prepubertally had an adverse effect on mammary development and inhibited cell proliferation. TCDD may exert its action via anti-estrogenic actions (7). No information is available on perinatal exposure to TCDD and the mammary gland. The goal of this study was

to determine whether TCDD given during an early period of development could alter mammary gland differentiation and cell proliferation and if it did, whether it would alter susceptibility to mammary cancer. The hypothesis to be tested was that an increase in TEBs (characterized as containing less differentiated terminal ductal structures) would be positively correlated with susceptibility to chemically induced mammary cancer.

Materials and methods

Animals, chemicals and treatments

Animal care and treatments were conducted in accordance with established guidelines and protocols approved by the UAB Animal Care Committee. Date bred Sprague–Dawley CD rats were purchased from Charles River Breeding Laboratories (Raleigh, NC). Upon receipt from the supplier, all animals were fed AIN-76A diet (Harlan Tex Lad, Madison, WI). AIN-76A is a semi-purified diet containing no detectable estrogens (limit of detection 5 mM). Diet and water were supplied *ad libitum*. Animals were kept in a climate-controlled room with a 12 h light/12 h dark cycle. The TCDD was obtained from Drs A.M Tritscher and G.W.Lucier (National Institute of Environmental Health Sciences, Bethesda, MD). Timed pregnant female rats were gavaged with 1 μg TCDD/kg body wt on day 15 post-conception or with an equivalent volume of sesame oil (~0.2 ml/animal). This dose has been reported to result in female offspring having altered uterine and ovarian estrogen receptor mRNA levels (26) and to cause subtle alterations in the endocrine and behavioral systems (27). These researchers did not study the mammary gland. Control animals were treated with the same volume of vehicle (sesame oil) on the same schedule. Within 10 h post-partum, offspring were cross-fostered to untreated dams (to minimize postnatal exposure). The offspring were sexed at birth and litters were reduced so that each dam was left with 10 offspring (4–6 females/dam). Weaning was carried out at day 21 post-partum. One randomly selected female from each litter was used at 21 and 50 days of age for the evaluation of mammary gland differentiation and cell proliferation.

Mammary gland differentiation

Abdominal glands (gland pair number 4) (33) were removed, one for preparation of the whole mount and the other for paraffin embedding and subsequent processing for labeling index measurements. While tumors developed in all of the mammary glands, gland pair 4 was analyzed because of ease of dissection. The whole mount was spread on a slide, fixed in 10% neutral buffered formalin (8–24 h), defatted in acetone (8–24 h), rehydrated in 70% ethanol (30 min), rinsed in water (15 min) and stained in alum carmine (2 g/l) overnight. After staining, glands were run through a series of graded alcohols (35–100% ethanol) and placed in xylene (24–48 h) to clear the tissue. Glands were then compressed between two glass slides for 24 h, released and allowed to expand for at least 8 h, then mounted using a glass coverslip and Permount (Fisher Scientific, Atlanta, GA). Coded whole mounts were evaluated via light microscopy using the criteria established by Russo *et al.* (11,34,35). The outer fringe of the mammary gland was evaluated (2.78 mm inward). This represents the location of most of the actively growing and carcinogen-susceptible terminal ductal structures in the gland (11,34,35). Whole mounts were evaluated for the number of TEBs, terminal ducts (TDs) and lobules type I and II. A TEB was designated as a club-shaped terminal ductal structure >100 μm in diameter which had three to six epithelial cell layers in the periphery of the bud. A TD had a diameter <100 μm and had one to three epithelial cell layers between the ductal lumen and outside edge of the structures. Lobules type I were comprised of 5–10 alveolar buds and lobules type II had 11–20 alveolar buds.

Mammary gland size

Mammary gland size was determined from whole mounts using an image analysis system linked to a video camera and a 486 computer. Carmine stained mammary glands were projected onto a video screen and prints were made using a Seikosha video printer. The perimeter of the gland was outlined on the video print and was traced by a sonic digitizer system (Graf Bar; Science Accessories Corporation, Southport, CT). The instrument records multiple x and y coordinates each second during a tracing and these coordinates are used in an in-house developed computer program to determine the area in mm^2. The system was calibrated with a micrometer photographed with the glands.

Cell proliferation

Labeling index measurements were done in the contralateral abdominal mammary gland using bromodeoxyuridine (BrdU) incorporation. Animals were injected with a single i.p. pulse of BrdU (100 mg BrdU/kg body wt dissolved in dimethylsulfoxide) 2 h prior to sacrifice. The contralateral abdominal gland was removed and placed in 10% neutral buffered formalin for 24 h. The tissue was embedded in paraffin, sliced into 5 μm sections and placed on SuperfrostPlus (Fisher) microscope slides. Tissue sections were deparaffinized in xylene and rehydrated using a series of graded alcohols (100–70%). The tissue sections were immersed in 3.6 N HCl for 15 min and then digested for 4 min using 0.01% trypsin in phosphate-buffered saline. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Slides were incubated for 20 min with 10% horse serum to block non-specific binding of the antibody, followed by a 30 min incubation (37°C) with BrdU primary antibody (Dako, Carpenteria, CA), a 20 min incubation (room temperature) with biotinylated horse anti-mouse secondary antibody (Signet, Dedham, MA) and a 20 min incubation (room temperature) with an Ultra Streptavidin detection system (Signet). Color was then developed with the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) and the tissue was counterstained with Gills no. 2 hematoxylin (Sigma). Tissue sections were evaluated using a Nikon light microscope and Sony video camera and monitor. All cell nuclei in each of three terminal ductal structures/gland were counted. Labeling index is defined as percent of cell nuclei incorporating BrdU (cell nuclei staining brown with DAB) divided by total cell nuclei counted. Hematoxylin was used as the counterstain.

Tumorigenesis

For the tumorigenesis experiments, 50-day-old female rats (30 prenatal sesame oil-treated and 29 prenatal TCDD-treated females) received 30 mg DMBA (in sesame oil)/kg body wt, via oral gavage. All animals were palpated twice weekly for mammary tumors until they were 230 days old, until the tumors reached 2.5 cm in diameter or until they were moribund. The location, relative size and date of appearance of each tumor were charted for each animal. Representative sections of each tumor were placed in 10% neutral buffered formalin. Tissues for microscopic examination were trimmed, embedded in paraffin, cut into sections 5 μm thick and stained with hematoxylin and eosin. Coded slides were evaluated histopathologically by Dr Roger Thompson, a board-certified veterinary pathologist.

Statistical analysis

Data from the tumorigenesis study was analyzed using the mathematical model proposed by Kokoska *et al.* (36). Standard analysis was conducted using the Wilcoxon rank sum test and the Fisher exact test. The Armitage test, as suggested by NCI guidelines (37), was used as an alternative perspective to the results obtained via the Kokoska approach. The analysis strategy followed for the mathematical modeling consisted of three parts. First, the distributional characteristics of the data were examined. Using the goodness-of-fit test suggested by Freedman *et al.* (38), the Poisson distribution was selected as the most appropriate model for the number of tumors per animal and the Weibull distribution was selected to describe the distribution of tumor appearance times. Second, the parameters associated with the model were estimated. The third step was to test the overall experimental effect of a change in the number of induced tumors and/or the time of tumor appearance (global test). Body and organ weights and mammary gland size, gland differentiation and cell proliferation data were analyzed by one way analysis of variance and Student's t-test using the Sigma Stat computer program (Jandel Scientific, San Rafael, CA).

Estrous cycle

Vaginal smears were collected from TCDD- and vehicle-treated animals on days 43–50 post-partum. The aspirate was collected and spread on a microscope slide, allowed to air dry and then Papanicolaou stained, with Orange G-6 (Surgipath, Richmond, IL), EA-50 (Surgipath) and hematoxylin (39,40). Stained slides were evaluated using a light microscope.

Results

Body and organ weights/size and sexual maturation

Prenatal TCDD treatment of dams on day 15 of gestation resulted in offspring with body weights significantly less than those from dams treated with the vehicle, sesame oil (control treatment), at 21 and 50 days of age (Table I). While liver weights of 21-day-old animals treated prenatally with TCDD and sesame oil were similar, 50-day-old TCDD-exposed animals had significantly lower liver weights as compared with the sesame oil-treated animals. Uterine weights and mammary gland size were not significantly different between TCDD-treated animals and control animals at 21 and 50 days of age. Investigations into sexual maturity revealed that prenatal TCDD treatment as compared with sesame oil resulted in

Table I. Body, liver and uterine weights and mammary gland size in 21- and 50-day-old female rats exposed prenatally to TCDD

Treatment (age)	Body weight (g)	Liver weight (g)	Uterus weight (mg)	Gland size (mm^2)
Sesame oil (21)	47 ± 1	1.7 ± 0.1	27 ± 4	30 ± 1
TCDD (21)	42 ± 1[a]	1.7 ± 0.1	22 ± 1	24 ± 3
Sesame oil (50)	199 ± 3	9.9 ± 0.3	316 ± 40	439 ± 13
TCDD (50)	176 ± 3[b]	8.5 ± 0.3[a]	237 ± 17	453 ± 10

Timed pregnant Sprague–Dawley CD female rats (8 per group) were gavaged with 1 μg TCDD/kg body wt on day 15 post-conception or with an equivalent volume of sesame oil (~0.2 ml/animal). Within 10 h post-partum, offspring were cross-fostered to untreated dams (surrogate mothers). One female from each litter was used at 21 and 50 days of age. Values represent means ± SEM.
[a]$P < 0.05$.
[b]$P < 0.001$ as compared with age-matched controls (sesame oil).

Table II. Estrous cycle in female rats exposed prenatally to TCDD

Treatment	Percentage of time spent in each phase of estrous			
	Proestrous	Estrous	Metestrous	Diestrous
Sesame oil	18/80 (22%)	17/80 (21%)	2/80 (3%)	43/80 (54%)
TCDD	15/80 (19%)	19/80 (24%)	2/80 (3%)	43/80 (54%)

Timed pregnant Sprague–Dawley CD female rats (8 rats/group) were gavaged with 1 μg TCDD/kg body wt on day 15 post-conception or with an equivalent volume of sesame oil. Daily vaginal smears (days 41–50 post-partum) were evaluated from the offspring (one from each litter). Values represent means ± SEM.

vaginal opening at 35 and 32 days respectively. Animals treated prenatally with TCDD, as compared with vehicle, spent more time in estrous (24 versus 21%, respectively) and less time in proestrous (19 versus 22%, respectively) (Table II). Nevertheless, all animals cycled.

Mammary gland differentiation

Whole mounts of the abdominal mammary glands were used to analyze the effects of TCDD on gland development. Figure 1A shows a whole mount of an abdominal mammary gland from an immature female rat. In Figure 1B are a TEB (upper structure) and a TD (lower structure). Figure 1C and D contains a lobule I and lobule II, respectively, from a 50-day-old female rat. The predominant terminal ductal structures of 21- and 50-day-old control female rats were TEBs and TDs (Table III). As the animals mature, TEBs develop into lobules I and then to lobules II (11,34,35). At 21 days of age there was no significant alteration in the number of terminal ductal structures due to prenatal TCDD treatment. However, prenatal TCDD treatment as compared with sesame oil treatment resulted in significantly more TEBs and fewer lobules II in 50-day-old animals.

Cell proliferation

Using BrdU incorporation and immunohistochemistry, we analyzed cell proliferation in the terminal ductal structures of the contralateral abdominal mammary glands. As seen in Table IV, the labeling index in 50-day-old female rats was higher in TEBs and TDs than in lobules I and II. Prenatal TCDD treatment did not significantly alter labeling index in TEBs, TDs or lobules of mammary glands of 21- and 50-day-old animals. However, when the increased number of TEBs in 50-day-old animals (Table III) was taken into consideration, the total proliferative compartment of TEBs from TCDD-treated animals was larger by 23%.

Tumorigenesis

Since prenatal TCDD treatment resulted in 50-day-old female offspring having an increased number of mammary TEBs and a larger proliferative compartment, we investigated chemically induced mammary tumorigenesis. Fifty-day-old female rats, treated on day 15 post-conception with either TCDD in sesame oil or with sesame oil only, were gavaged with 30 mg DMBA/kg and subsequently palpated for tumors. Female rats treated prenatally with TCDD developed twice as many tumors as compared with female rats treated prenatally with sesame oil only (4.475 ± 1.582 versus 2.127 ± 0.340, $P < 0.001$; Figure 2). The mean times to tumor detection were 154 ± 69 and 111 ± 43 days in TCDD- and sesame oil-treated animals, respectively. Animals treated with TCDD and DMBA showed 90% tumor incidence, while animals treated with vehicle and DMBA developed a 79% tumor incidence. Sixty-one percent of the mammary tumors developed in the thoracic region, while 31 and 8% were located in the abdominal and inguinal regions respectively. Histopathological evaluation of mammary tumors revealed that all animals with tumors had at least one adenocarcinoma.

Discussion

The mammary gland has, for the most part, been overlooked as a target organ for biochemical insult as a consequence of exposure to environment pollutants. Of the limited animal studies that have been carried out, few have paid attention to limited periods of development. For our initial studies, we selected TCDD because dioxin has been listed as a potential carcinogen (2,41). However, for every report of dioxin being associated with breast cancer there seems to be one that finds no significant effect (2–7). We selected one early critical period of development for exposure because there is evidence that exposure to certain hormonally active chemicals during differing periods of development can result in opposite predispositions to mammary cancer (12–15,32). For this study, we chose the prenatal period of development. The dose selected was based on reports of TCDD causing slight but significant developmental alterations to the mammary gland and liver and endocrine and behavioral systems (7,26,27). To minimize

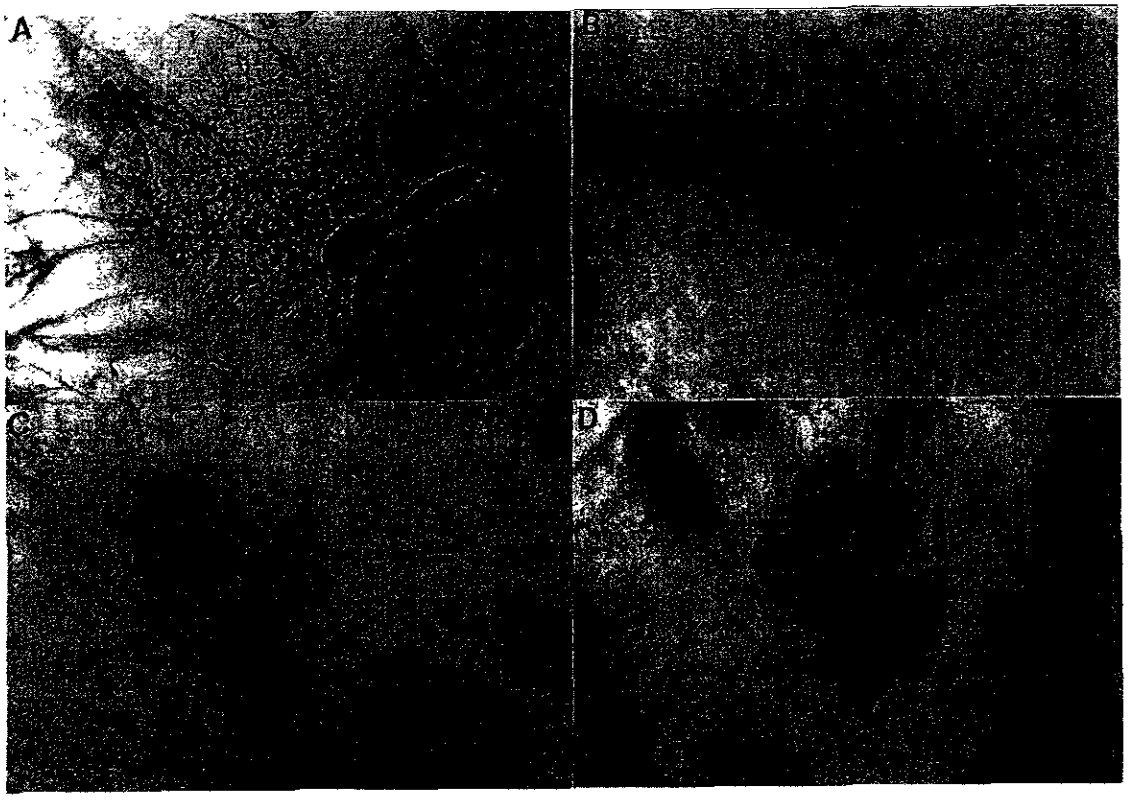

Fig. 1. Terminal ductal structures in a rat mammary gland. (**A**) Whole mount of a fourth abdominal mammary gland from a prepubertal female rat. Note nipple at upper left corner and lymph nodes at botton right. (**B**) The upper structure is a TEB, the lower structure a TD (**C**) Lobule I. (**D**) Lobule II.

Table III. Number of mammary terminal ductal structures in female rats exposed prenatally to TCDD

Treatment (age)	Terminal ductal structures			
	TEB	TD	Lobules I	Lobules II
Sesame oil (21)	42 ± 8	50 ± 4	6 ± 2	[a]
TCDD (21)	54 ± 4	43 ± 9	3 ± 1	[a]
Sesame oil (50)	111 ± 1	120 ± 12	74 ± 4	22 ± 6
TCDD (50)	149 ± 1[b]	111 ± 8	80 ± 6	8 ± 2[b]

Timed pregnant Sprague–Dawley CD female rats (8 per group) were gavaged with 1 μg TCDD/kg body wt on day 15 post-conception or with an equivalent volume of sesame oil. One offspring from each litter was evaluated. Values represent the means ± SEM.
[a]Insufficient number of structures to quantify Values represent means ± SEM.
[b]$P < 0.05$ as compared with age-matched controls (sesame oil)

Table IV. Cell proliferation in mammary terminal ductal structures of rats exposed prenatally to TCDD

Treatment (age)	Labeling index			
	TEB	TD	Lobules I	Lobules II
Sesame oil (21)	23.4 ± 3.1	22.4 ± 3.1	[a]	[a]
TCDD (21)	20.9 ± 1.8	21.8 ± 2.4	[a]	[a]
Sesame oil (50)	22.0 ± 2.4	22.2 ± 1.9	3.1 ± 0.7	1.8 ± 0.8
TCDD (50)	20.1 ± 2.0	25.1 ± 2.5	5.0 ± 1.9	2.6 ± 0.8

Sprague–Dawley CD rats were exposed prenatally to TCDD or vehicle. Two hours prior to sacrifice, one offspring from each litter was injected (i.p.) with 100 mg BrdU/kg body wt. Labeling index was determined in three of each of the terminal ductal structures/gland (8 rats/group). Values represent the means ± SEM in each terminal ductal structure.
[a]Insufficient number of structures to quantify.

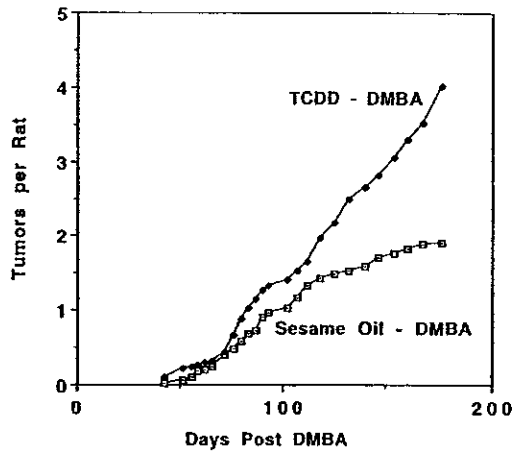

Fig. 2. Ontogeny of palpable mammary tumors in female rats exposed prenatally to TCDD and treated with DMBA on day 50 post-partum. Pregnant female Sprague–Dawley CD rats were treated with 1 μg TCDD/kg body wt or an equivalent volume of the solvent (sesame oil) on day 15 post-conception. DMBA was administered by gavage at 30 mg/kg body wt to 50-day-old female offspring. The TCDD/DMBA group and the sesame oil/DMBA group contained 29 and 30 female rats/group, respectively.

postnatal exposure, all offspring were transferred to untreated surrogate mothers shortly after parturition. However, due to the biological persistence of these xenobiotics, it is impossible to clear TCDD from the animals.

Body and organ weights/size and sexual maturation

Prenatal TCDD exposure resulted in reduced body weight at 21 and 50 days and reduced liver weight at 50, but not at 21, days of age. Prenatal TCDD exposure also resulted in a delay in vaginal opening and an altered estrous cycle, even though these animals did cycle. Others have reported similar findings for perinatal TCDD exposure (26,27).

Mammary gland differentiation and cell proliferation

Our results demonstrate that the mammary glands of developing rats are sensitive to prenatal TCDD treatment. However, the effect is subtle. Prenatal exposure to TCDD did not significantly affect gland size or cell proliferation at the ages investigated. It did alter mammary gland differentiation in the adult animals, but not in the weanling animals. This is an effect of significant physiological importance. In adult animals, an increase in number of TEBs and a decrease in number of lobules II is characteristic of a less differentiated mammary gland. From a standpoint of susceptibility to mammary cancer, the increase in number of TEBs is crucial. We (13,14) and others (34,35) have shown that there is a direct correlation between number of TEBs and susceptibility to chemically induced mammary cancer. TEBs are the least mature and most susceptible terminal ductal structures, while the lobules are more mature and less susceptible structures to chemical carcinogens. Furthermore, prenatal TCDD treatment, as compared with control treatment, resulted in TEBs in 50-day-old animals having a 23% larger labeling compartment. The increased number of TEBs in TCDD-treated animals as compared with control animals largely accounts for this proliferative compartment. The fact that gland differentiation was not altered at day 21 post-partum, but was at day 50, argues against residual TCDD concentrations being responsible for the alterations to mammary gland differentiation. The increased number of TEBs and decreased number of lobules II may occur as a consequence of altered cellular programing mechanisms and/or hormone action. Alterations to programing mechanisms in the mammary gland and liver and endocrine and behavioral systems due to perinatal exposure to estrogenically active xenobiotics have been reported (12–15,42–44). Programing or imprinting mechanisms are hypothesized to be set early in development and to determine the program by which cells respond later in life to external (and internal) stimuli. Hence, hormonally active chemicals such as estradiol-17β, diethylstilbestrol, genistein, PCBs and TCDD, could cause programing effects during early development, yet they will not be evident until after puberty. In this way, in the absence of the original programing effector, a stimulus (sex hormones, growth factors or environmental agents) could interact with the target organ and the program will be used to regulate gene expression, etc.

Tumorigenesis

Since we found more TEBs and a larger proliferative compartment in offspring of female rats treated prenatally with TCDD, we hypothesized that these adult animals would be more susceptible to mammary cancer. Hence, we investigated the potential of prenatal TCDD treatment to enhance chemically induced mammary cancer using the DMBA/rat model (45). For this we used a DMBA concentration that yielded a low number of tumors in control animals to facilitate observation of an increase in tumorigenesis but still yield adenocarcinomas. We determined this to be a minimum of 30 mg DMBA/kg body wt (unpublished data).

Adult female rats treated prenatally with TCDD and then with DMBA at day 50 post-partum developed twice as many tumors as compared with adult female rats treated prenatally with vehicle only and then with DMBA. The TCDD/DMBA animals also developed a higher tumor incidence and had a shorter mean time to tumor appearance than control/DMBA animals. Historical data from our laboratory revealed that female Sprague–Dawley CD rats not treated with DMBA do not typically develop adenocarcinomas. A 2 year chronic toxicity study with TCDD did not report increased incidence of mammary cancer (46).

Timing of exposure to endocrine disruptors can influence the ultimate outcome of a biochemical insult. TCDD given to rats 3 days prior to administration of DMBA has been reported to lengthen the time required for development of DMBA-initiated mammary tumors in female Sprague–Dawley rats (6). This is presumably due to the direct induction of specific cytochromes P-450 that can metabolize genotoxic carcinogens (47). Also, TCDD administered to rats having DMBA-induced mammary tumors was able to inhibit growth of these tumors. This may be due to the anti-estrogenic and anti-promotional actions of TCDD (7). It was also reported that diethylstilbestrol given prenatally enhanced chemically induced mammary tumors (32), while neonatal diethylstilbestrol treatment resulted in a decrease in spontaneously developing mammary tumors (15). Even the weakly estrogenic isoflavone genistein programs against chemically induced mammary cancer when exposure takes place postnatally (12–14). The rate of formation and persistence of carcinogen–DNA adducts was not responsible for genistein chemoprevention (48). In the mammary gland it appears that differentiation plays an important role in susceptibility to or protection against cancer. While mammary gland morphology has been identified as a cellular marker that can be used to predict cancer susceptibility, cell differentiation

within the terminal ductal structures appears to be specific at the molecular level. Recently, we discovered that prepubertal genistein exposure programs for reduced expression of the EGF signaling pathway (49).

In humans, neither ecological data nor occupational studies provide clear support for an association between organochlorine endocrine disruptor exposure and occurrence of breast cancer (2,5). This may be explained by the presence of only very low concentrations in humans, the dual effects of their properties (estrogenic/anti-estrogenic) and/or timing of exposure. It is possible that postnatal, as opposed to prenatal, exposure to TCDD may yield a different outcome, perhaps rendering a protective effect against mammary cancer. It is our intention to investigate the potential of neonatal TCDD treatment to predispose for mammary cancer and the underlying molecular mechanism of action of perinatal exposure to organochlorines. Furthermore, we believe that exposure to/treatment with the same agent during two periods of development could modify the individual predisposition.

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